Mapping *sdm328*, a Suppressor of *dmc1-2* in *Arabidopsis thaliana*

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Abstract

In meiosis, diploid progenitor cells divide to produce haploid gametes, during which chromosomes exchange genetic material. This is known as meiotic recombination and is essential for proper chromosome segregation. The recombinase DMC1 assists in strand invasion between homologous chromosomes during recombination in the plant species *Arabidopsis thaliana*. A forward genetic screen for reduced recombination frequency resulted in identification of a hypomorphic allele of *DMC1*, *dmc1-2*. Additional mutagenesis revealed candidate suppressors that possibly interact with DMC1; these suppressors ameliorate the partial-sterility phenotype caused by the *dmc1-2* allele. This project seeks to map the suppressor *sdm328*. I grew and genotyped 90 F_2s from a cross between Columbia (*dmc1-2*$^{-/-}$; *sdm328*$^{-/-}$) and Landsberg erecta (*DMC1*$^{+/+}$; *SDM328*$^{+/+}$), revealing 19 *dmc1-2* homozygotes that were scored for fertility. Ten polymorphic markers between Columbia and Landsberg erecta were used to genotype the F_2s suggest that sdm328 is on the bottom arm of Chromosome 1. A pool of fertile and sterile F_2s will be submitted for Illumina sequencing to genetically map the *SDM328* locus. Our findings will contribute to current knowledge of meiotic recombination and proteins that interact with DMC1.

INTRODUCTION

Meiosis is a biological process in which diploid germline progenitor cells divide their genomes to produce haploid gametes (sperm and eggs). During meiosis, crossovers (reciprocal exchange of genetic material between homologous chromosomes) are formed in a process called homologous recombination (HR). By creating physical connections between homologous chromosomes, proper pairing and segregation of homologous chromosomes can occur; crossovers are necessary in most sexually-reproducing organisms [1]. HR can lead to the formation of crossovers, non-crossovers (no exchange of DNA), or non-crossovers with gene conversions (sequence information is exchanged between homologous chromosomes without physical exchange of DNA) [1].

There are two main models for meiotic recombination (Figure 1); the Double-Strand Break Repair model (DSBR) and the Synthesis-Dependent Strand-Annealing model (SDSA) [6]. In the DSBR model, the first step is the production of a DNA double-stranded break (DSB) in one chromatid catalyzed by the highly conserved SPO11 protein and its accessory proteins [7]. After the DSB has been made, the exposed 5′ ends are resected, leaving single-stranded 3′ tails. One of the single-stranded tails then invades a homologous chromatid, creating a displaced strand called a D-loop. The invading end is used to prime a short stretch of DNA synthesis which extends the D-loop. D-loop extension enables it to capture the second 3′ tail on the other side of the DSB. A double Holliday Junction (dHJ) intermediate is created when each of the exposed ends are ligated to the other. Formally, it is possible to process dHJs to produce either a crossover or a noncrossover. However, based on work in yeast, it is

thought that dHJ resolution produces primarily (if not exclusively) crossovers [7]. Noncrossovers are thought to come primarily from the SDSA pathway [8]. SDSA begins in the same manner as DSBR but instead of successfully completing second-end capture, the invading 3′ tail dissociates from the extended D-loop and re-anneals to the complementary tail on the other side of the DSB to form a noncrossover [4].

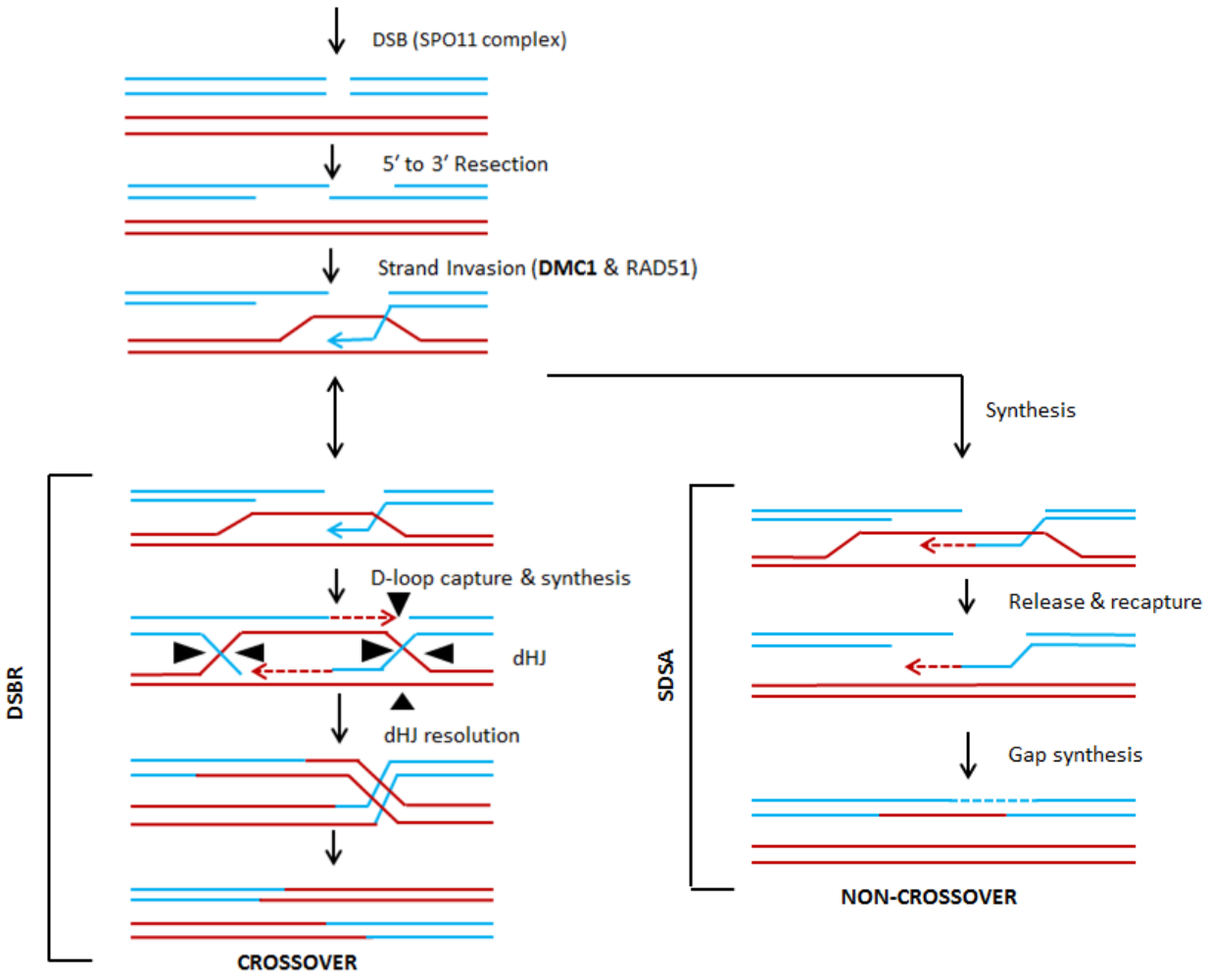

Figure 1: The Double Stranded Break Repair (DBSR) and Synthesis-Dependent Strand-Annealing (SDSA) models of meiotic recombination that can lead to either crossovers or

noncrossovers, respectively. The blue and red strands represent the interacting homologous chromosomes.

The recombinase DMC1 (Disrupted Meiotic cDNA 1 [12]) assists in strand invasion between homologous chromosomes during recombination. In the DSBR model, the 3′ single-stranded tail of DNA must find a homologous DNA sequence. This process is mediated by DMC1 and another DNA recombinase, RAD51 [2]. DMC1 is meiosis-specific and works with RAD51 to form presynaptic filaments after binding to the single-stranded tails. As RAD51 functions in both mitotic and meiotic recombination, it is thought that DMC1 assists in the preference for non-sister over sister chromatids. Without the functional DMC1 protein, DSBs are repaired by RAD51 using the sister chromatid, resulting in a lack of crossovers. Although mutants have repaired DSBs, they fail to form a normal synaptonemal complex (SC). In WT plants, the SC forms along the entire length of paired homologous chromosomes during meiotic prophase, elongating as the DSBs disappear. The absence of crossovers in *dmc1* null mutants causes an abnormal but complete meiosis with 10 univalents that fail to segregate properly, leading to sterility [12, 13].

DMC1 or its homologs are found in bacteria, fungi, plants, and animals. There is one copy of the gene in Arabidopsis, though other plants, like bread wheat, have multiple [10]. The DMC1 protein is a structural and evolutionary homolog of the bacterial protein RecA, which assists in processes such as conjugation, bacteriophage-mediated transduction, plasmid recombination, and DNA damage repair. *DMC1* was identified in *Saccharomyces cerevisiae* in a screen for genes that had meiosis-specific expression patterns [12]. In S. cerevisiae there are at least ten accessory proteins that are thought

to interact functionally or physically with DMC1 [14}. The proteins that assist DMC1 in its strand-exchange activity are not well characterized, with Arabidopsis only sharing four of the ten proteins in *S. cerevisiae* that interact with DMC1 [12]. The broad objective of this research project is thus to identify proteins that interact with DMC1, furthering our understanding of the essential role of DMC1 in meiosis.

In a forward genetic screen for reduced recombination frequency, the Copenhaver lab found a hypomorphic allele of *DMC1*, *dmc1-2*, which was induced by ethyl methanesulfonate (EMS) mutagenesis. Through additional EMS mutagenesis, several mutants have been identified that act as suppressors of the partial-sterility phenotype caused by a *dmc1-2* allele, leading to a reversion to full fertility [11]. The long-term goal of this project is to map and characterize the *DMC1* suppressors and determine to what extent they can mitigate defects in DMC1 and other recombinases. My project specifically aims to genetically map *sdm328*, a recessive suppressor of *dmc1-2*.

METHODS

Plant growth

A *dmc1-2*$^{-/-}$; *sdm328*$^{-/-}$ plant in the Columbia background was crossed to Landsberg erecta (*DMC1*$^{+/+}$; *SDM328*$^{+/+}$) in order to produce F_1 plants. F_1 seeds were sterilized with a 70% ethanol solution followed by a 50% bleach solution, then rinsed with sterile distilled water three times. Seeds were then sown on MS-Agar plates for germination. After 4 days at 4°C for stratification, the plates were moved to a Percival growth chamber with an 18h day length at 22°C. The seedlings were transferred to

individual 4” pots filled with MetroxMix 360 potting soil after two weeks and subsequently moved into a 21°C growth room with an 18h day length. F_2 seeds from a single F_1 plant (*dmc1-2*$^{+/-}$*; sdm328*$^{+/-}$) were sown in 90 individual 2.5” pots in three flats. Each flat also had a Columbia (*DMC1*$^{+/+}$; *SDM328*$^{+/+}$) plant and a *dmc1-2 (dmc1-2*$^{-/-}$; *SDM328*$^{+/+}$) plant as controls. The seeds were stratified for 4 days at 4°C before they were transferred into a 21°C growth room with an 18-hour day length.

DNA Extraction

Once the true leaves emerged, a leaf was collected, placed in 400µL of Edward’s buffer (200 mM Tris, pH 8.0, 200mM NaCl, 25 mM EDTA, 0.5% SDS), and ground with a sterile pestle. To extract the DNA, samples were placed in a centrifuge at 14000rpm for 5min. 300µL of the supernatant was transferred to a fresh Eppendorf tube. Then, 300µL of isopropanol was added and the tube inverted 10 times to ensure thorough mixing. After a 2 minute incubation period at room temperature, the sample was centrifuged for 5min at 14000rpm. The supernatant was discarded and the pellet was washed with 700µL of 70% ethanol before centrifuging for 2 minutes. The supernatant was discarded and, the DNA pellets were air dried for 30min. Samples were resuspended in 100µL of sterile distilled water and stored at 4°C.

DMC1 Genotyping using Derived Cleaved Amplified Polymorphic Sequences (dCAPs)

A dCAPs (derived cleaved amplified polymorphic sequence) primer set was used to genotype F_1 and F_2 plants at the *DMC1* locus [15]. The DNA was amplified by PCR in a 15µL reaction containing 7.5µL of APex taq DNA polymerase mix, 3.3µL distilled water, and 0.35µL of forward and reverse dCAPs primers to amplify a 348 base pair

region of *DMC1*. The sequence for the dCAP forward primer sequence is 5′ - GCAAGTTGTACTGATGCTCATAGGTTAA - 3′, and the reverse primer sequence is 5′ - CATGAAAGGAGGGAATGGAA - 3′. These primers incorporate a *Pme*I cut site into the mutant allele. *Pme*I recognizes and cuts the mutant allele at one location, creating fragments of 321 base pairs and 27 base pairs, allowing wild-type and mutant alleles to be separated on an agarose gel. The DNA was digested by the restriction enzyme *Pme*I in a 30μL reaction using 1μL of the *Pme*I enzyme, 3μL of NEB CutSmart buffer, 16μL of distilled water, and 10μL of PCR reaction. 3% agarose, 100 mL and 200mL gels were used with 1x10TBE buffer and ethidium bromide run at 150 volts for 110 minutes in order to genotype the construct. The Generuler 1kb Plus DNA ladder was used for reference.

Fertility Analysis

After the *dmc1-2* homozygotes were identified from the F_2 mapping population, siliques 5-9 were harvested from the primary meristem and scanned as transparent images using an EPSON scanner (EPSON Perfection V550 Photo). The number of seeds in each silique was counted manually and the length of each silique was measured in the program ImageJ. Additionally, a qualitative visual fertility assessment was used to determine the level of fertility and whether the plants phenotypically resembled either the Columbia or Landsberg erecta parental strains. Fertility was ranked on a scale of 1-3 with plants exerting a very sterile phenotype (e.g. small, empty siliques) being labeled as a 1 and plants displaying a very fertile phenotype (e.g. large, full siliques) being labeled as a 3. Plants that displayed a partial-sterility phenotype were labeled as a 2.

SSLP Marker Analysis

Simple Sequence Length Polymorphism (SSLP) markers that are polymorphic between Columbia and Landsberg erecta were used to genotype the F_2s. DNA from each F2 and the primers listed in Table 1 were used to amplify the markers in a 15μL reaction containing 7.5μL of APex taq DNA polymerase mix, 3.3μL distilled water, and 0.35μL of the forward and reverse primers. Samples were then run on two 200mL 3% agarose gels with 1x10TBE buffer and ethidium bromide run at 150 volts for 90 minutes. Col and F_1 controls were used in order to distinguish between Col and Ler alleles. A Tukey-Kramer HSD test was used to test for a difference among means between the genotypic classes for markers with significant ANOVAs ($\alpha < 0.10$). All analyses were performed in R (3.1.2, The R Foundation for Statistical Computing).

Table 1.SSLP Marker Primer Sequences

Marker	Chromosome	Forward Primer Sequence (5′-3′)	Reverse Primer Sequence (5′-3′)
8002-03		ACCAACACCACAACAAACGAC	CTTTTTCTGTTCTTCCGCTATTC
8014-15	1	GATACGTTCAAAATTAGGGACTTC	TGTATTTTGCTAATTGAGGTTATGG
8018-19		CTGGAGATCATCCAACAAAG	TGCAATGGAATGGGCTGGTC
8028-29	2	CGGGCTGTCCATGAACTATT	AAACCAAATCGAACCAACCA
8036-37		CCCTGGCAAGACATAACCAA	CTTCTTGTTTTGCCTCTGTGG
8044-45	3	TGGACCCAAGTCTTTGGATT	CCCTCGTTTCTCTTTCTCGTT
8052-53		TGAAGCGGTTTCAAGATTGTT	CGCAAACTATTTGCCATAGTCA
8060-61	4	AGAGAGCACGATGCCTGATAG	AATGCTTCAGCGATTGAGAAC
8070-71		CGTTATGTAATAGTCATCACGTTTTTG	TTCACAATTAGAACGCTGAATCAT
8080-81	5	TGAACTGGATCAGCTTTACTTGA	AGGTGCACGAGATGGTTCAT

RESULTS

Thirty F_1 30 seedlings were grown and genotyped at the *DMC1* locus. The results of the F_1 genotyping are displayed in Figure 2, showing primarily heterozygotes (8 *dmc1-2*$^{+/-}$, 5 *dmc1-2*$^{-/-}$, 17 showed no amplification), as indicated by the two prominent bands at 348 and 321bp in each lane. The wild-type allele is 348 base pairs, while the mutant allele was cut with the *Pme*I enzyme into two pieces of 321bp and 27 bp.

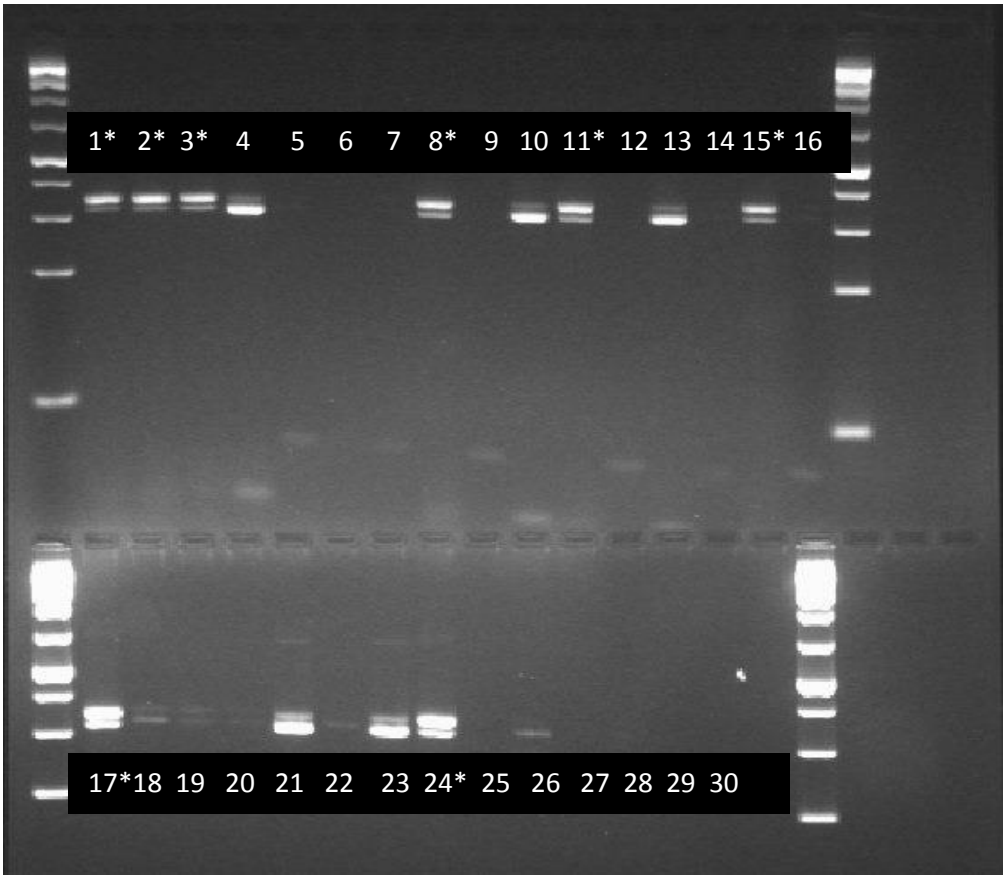

Figure 2. F_1 plants run on a 3% agarose gel using 1xTBE buffer and a Generuler 1kb Plus DNA ladder for reference.

The F_1 plants were self-fertilized and the seeds were collected. Seed from a single F_1 was used to create the F_2 mapping population. 90 F_2 plants were grown and genotyped to determine which plants were homozygous for the *dmc1-2* mutation, as indicated by a distinct 321bp band. The results from the F_2 genotyping at *DMC1* are displayed in Figure 3, which revealed 19 *dmc1-2*$^{-/-}$ F_2 plants. As there were 45 heterozygotes and 26 WT plants, the F_2 generation displays the expected 1:2:1 segregation rates (the deviation is not significant, p-value=0.297).

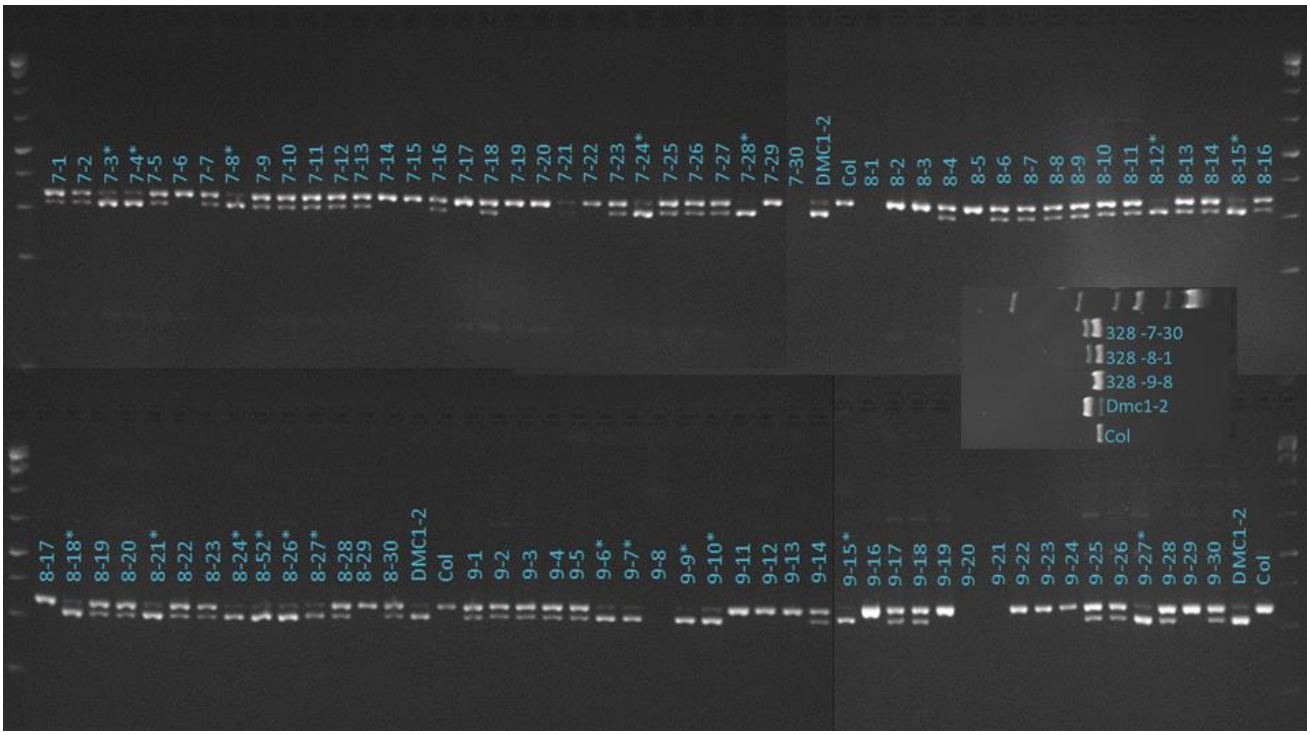

Figure 3. F_2 plants run on a 3% agarose gel using 1xTBE buffer and a Generuler 1kb Plus DNA ladder for reference. Samples names are labeled above the band and *dmc1-2*$^{-/-}$ F_2 plants are indicated with a '*'.

Using average seed count among siliques as the measure for fertility, the F_2s were compared to *dmc1-2*$^{-/-}$*; sdm328*, *dmc1-2*, and Columbia controls. Results are displayed in Figure 4. The F_2 population is segregating for fertility, but displays a primarily partial-sterile phenotype.

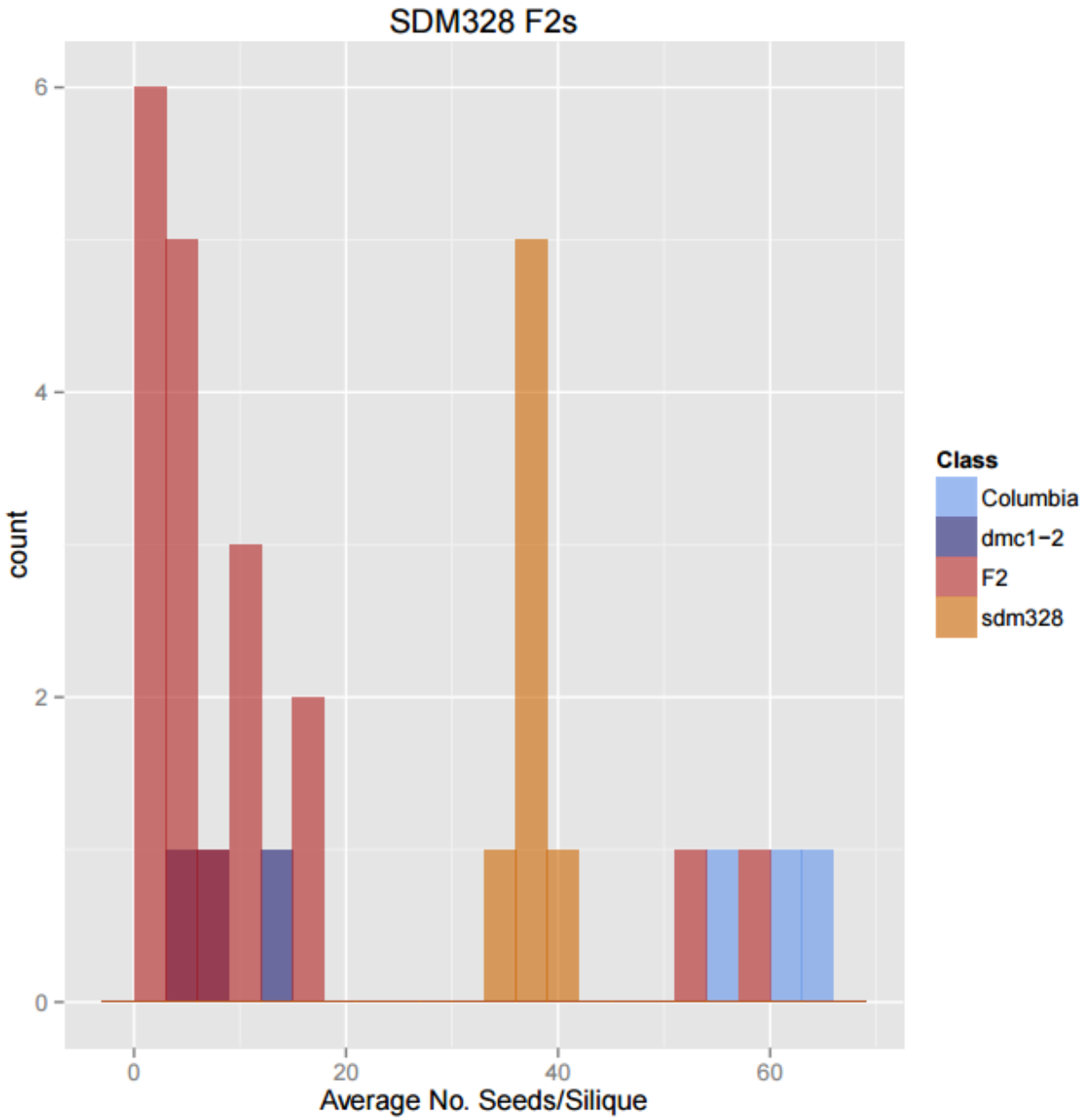

Figure 4. The average number of seeds per silique among the *dmc1-2*$^{-/-}$ F_2 population and control populations. Markers for the *dmc1-2* homozygotes appear as either dark blue or as dark maroon (where there is overlap).

Four fertile plants (plants with seed counts higher than the *dmc1*-2 plants) were chosen for the fertile pool and four sterile plants for the sterile pool. These pools will be submitted for Illumina sequencing after a CTAB DNA extraction.

As a preliminary measure to help determine the location of the sdm328 locus, a set of ten simple sequence length polymorphism (SSLP) markers that are polymorphic between Columbia and Landsberg erecta were used to genotype the F2s. Each of the five Arabidopsis chromosomes had two markers, one for each chromosome arm. As the smd328 allele was derived from the Columbia background, the chromosome arm containing the sdm328 gene should be linked to a Columbia marker. Fourteen F_2 plants (seven with the highest and seven with the lowest seed counts), a Columbia and F1 control, were amplified using the primer sequences listed in Table 1. A one-way ANOVA between Columbia allele frequency for the 8014-8015 SSLP marker and the qualitative fertility assessment for each plant was significant ($F_{1, 12} = 14.453$, p-value =0.0007825); this marker is on the bottom arm of chromosome 1. A Tukey-Kramer HSD test revealed that the Columbia homozygotes (M=2.60) did have a significantly higher qualitative fertility assessment than the Ler homozygotes (M=1.50) for the 8014-8015 marker. The difference between the Columbia homozygotes and heterozygotes (M=2.31) was not significant. F_2s genotyped at the 8036-37 marker on the top arm of chromosome 3 were all Columbia homozygotes, as this marker is likely linked to *DMC1*. The gel for the SSLP markers is displayed in Figure 5.

Figure 5. Seven fertile and seven sterile F_2 plants with SSLP primers run on a 3% agarose gel using 1xTBE buffer and a Generuler 1kb Plus DNA ladder for reference. Samples are labeled above the gel, with the marker labeled underneath.

DISCUSSION

Eight *dmc1-2*$^{+/-}$ heterozygous F_1 plants were identified. The five *dmc1-2* mutant plants are likely the result of pollen contamination from the maternal plant (*dmc1-2*$^{-/-}$; *sdm328*$^{-/-}$). Seed from a single self-fertilized F_1 was grown, and genotyping of F_2s

revealed 19 homozygous mutants. Plants were sorted into fertile (presumably *dmc1-2*$^{-/-}$; *sdm328*$^{-/-}$) and sterile pools (presumably *dmc1-2*$^{-/-}$; *SDM328*$^{+/+}$ or *dmc1-2*$^{-/-}$; *sdm328*$^{+/-}$), based on the phenotypic assessment and pooled DNA from each of these pools will be Illumina sequenced. Four fertile plants with average seed counts higher than the *dmc1-2* plants made up the fertile pool. As expected the majority of the F_2 plants were exerting the partial-sterility phenotype, as the *sdm328* allele is recessive. Four plants with very low seed counts were chosen for the sterile pool. To ensure high quality DNA, a CTAB DNA extraction will be used to extract DNA for the fertile and sterile pools.

Data from the SSLP marker analysis suggests that *SDM328* is potentially on the bottom arm of chromosome 1. Additional F_2s and marker analysis including a denser set of markers, as well as Illumina sequencing, will assist in narrowing down the region on the bottom chromosomal arm. Marker 8070-71 was removed from consideration based on preferential amplification of the Columbia allele in heterozygous plants.

Allele frequency differences between the pools will be calculated from the Illumina sequencing data and used to identify the genomic location of *SDM328*. Because the *dmc1-2* allele is originally derived from a Columbia (Col) background, and was crossed to Landsberg erecta (Ler), the entire F_2 population should have a 50:50 ratio of Col to Ler alleles in regions that are unlinked to *SDM328*. However, in the fertile pool (presumably *dmc1-2*$^{-/-}$; *sdm328*$^{-/-}$), F_2s should be homozygous for Columbia-derived alleles at the *SDM328* locus, thus allowing us to pinpoint its location in the genome.

Additional F_2 populations will be needed in order to present stronger statistical differences before submission for sequencing. After the identity of the *SDM328* gene is known, we shall further characterize its molecular interactions and ability to mitigate recombinase defects by looking at aspects such as whether an *sdm328* mutant will influence crossover frequency without *dmc1-2* in the background and whether it can compensate for defects caused by RecA homologs other than DMC1, like RAD51. Then, we shall investigate the other potential suppressors uncovered by the EMS mutagenesis screening. By determining the genomic location and role of *SDM328* as a suppressor of *dmc1-2*, we are contributing to knowledge of the proteins interacting with DMC1 and the process of meiotic recombination.

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